EVALUATION OF A NOVEL BLEND OF PREBIOTIC AND PROBIOTIC COMPOUNDS WITH CHELATED MINERAL ADDITIVES ON BREEDING BULL GROWTH AND FERTILITY

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DIONICIO GUTIERREZ, Jr.

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DIONICIO GUTIERREZ, Jr.

APPROVED:

Dr. Chase A. Runyan

Dr. Cody Scott

Dr. John Kellermeier

Dr. Ned Strenth

May 2021

APPROVED:

Dr. Micheal W. Salisbury
Dean, College of Graduate Studies and Research

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ABSTRACT

The purpose of this study was to evaluate the effects of prebiotic and probiotic compounds with chelated mineral additives (COMBO) that was manufactured for feedlot cattle health and growth, on breeding bull growth and fertility. The 2019 spring-born bull calves (n=23) were weaned and stratified by age of dam (AOD) and weaning weight into one of two groups, the control group (n=11) or the treatment group (n=12). The same dietary ration was provided for both groups except for the ASU pre-mix additive in the control group being substituted with the COMBO additive in the treatment group. Weight and scrotal circumference were taken once a month during the trial period and semen was collected on the last measurement day for a semen quality test. The measurements were assessed on day 58 and day 115 of the research period. The treatment groups average daily gain (ADG) showed improvement to day 58 then maintained a steady growth as the control ($P \leq 0.05$). Scrotal circumference up to day 115 was less ($P \leq 0.05$) with the control having better results. The semen testing concentration did not seem to be of great concern, but the semen motility and progression were less ($P \leq 0.05$) making the semen sample of undesirable quality. The measurements were analyzed with the impact of the diet and an injectable trace mineral (ITM). The results indicate that feeding the COMBO additive had an unfavorable impact on bull growth and fertility.

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INTRODUCTION

Breeding bulls are a necessity in the cattle industry, whether it be through natural service or semen for artificial insemination. Today, through properly combining feedstuff rations, cattle can take advantage and utilize nutrition more efficiently and effectively for growth and development. In the production of commercial cattle and breeding herds, there is a significant amount of information on nutrition and additives regarding cows, heifers, and steers. But limited information exists regarding breeding bulls and combined additives.

Additives like yeasts, bacterial prebiotics or probiotics, and chelated trace minerals have been found to have different outcomes in the ruminant's digestive system. Yeasts are known to improve rumen function and digestibility, bacterial prebiotics helps keep the rumen bacteria in balance, and trace minerals help supply any mineral deficiencies (Cagle et al., (2018), Uyeno et al., (2015), Rowe et al., (2014)). Currently, these additives are given as individual supplements or can be combined. COMBO is a proprietary feed additive that is manufactured as a premix that can be added to feed rations.

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OBJECTIVE

During the trial period, experimental units were monitored for weight gain, anatomical reproductive growth, and sperm cell quality measures. The recorded data from these areas was used to determine if the incorporation of the COMBO additive has improvements in the stated areas. The study of growth in the reproductive anatomy in pre-pubertal breeding bulls was beneficial since currently there is limited publication regarding this topic.

The objective of this project was to evaluate the effects of a proprietary blend of prebiotic and probiotic compounds with chelated mineral additives on breeding bull growth and fertility measures.

LITERATURE REVIEW

There are several prebiotic/probiotics, yeasts, and chelated minerals, and the research for these additives has produced a variety of results (Cagle et al., 2018, Uyeno et al., 2015, Rowe et al., 2014, Beauchemin et al, 2003). The use of these additives on lactating dairy cattle, the reproduction in cow/calf operations, and the growth and development of feedlot cattle have questioned if these additives have significant effects that bring value to the industry. Many of these studies results (Cagle et al., 2018, Uyeno et al., 2015, Rowe et al., 2014, Beauchemin et al, 2003), have used one or a combination of these additives. Because many of the formulated combinations of prebiotic/probiotic, yeast, and chelated mineral products are designed to improve weight gain and enhance beef carcass characteristics, much of the fertility aspects of these products remain unknown. Therefore, this project was designed to better understand the interacting nature of these additives and to better quantify their impacts on growing bull performance and spermatogenesis.

BREEDING BULLS

The development of breeding bulls is extremely important for any breeding bull producer, especially from the time of birth until breeding or semen collection. Knowing the stages when a bull needs an adequate amount of nutrition is essential. When a calf is weaned up to puberty is one of those stages where proper supplementation is important, to ensure that the breeding bull is at its best potential, these requirements need to be met. When not being met growing bulls can exhibit reduced muscle growth as well as permanently impaired sperm production (Hafts et al., 1959: Van Demark and Mauger, 1964). As nutritional requirements for maintenance and growth are met, additional nutrient intake can be allocated to

reproductive development. Puberty is defined as the time at which a young bull can produce semen with 50 × 106 spermatozoa/mL and at least 10% of the sperm are progressively motile (Arteaga et al., 2001). A well-nourished bull can develop a reproductive system easier as it reaches puberty. Bulls consuming a higher quality protein diet had greater measures of body weight, scrotal circumference (SC), total sperm motility, and higher ejaculate concentrations in bulls after 12-14 months of age (Barth et al., 2008).

Engelken (2008) mentions that there are heritable traits that influence fertility; one of those is scrotal circumference with a heritability range of 0.36 to 0.68. Certain fertility measures can be associated with scrotal circumferences like predicting puberty, percent sperm motility, normal sperm, sperm volume and concentration, total sperm output, and total epididymal sperm reserves which are all favorable in a good and reliable bull (Engelken, 2008). Despite the heritability of scrotal circumference, proper nutrition is essential for proper scrotal circumference (Engelken, 2008). Thus, providing a well-formulated feed ration, including a reliable premix can be essential for proper reproductive development.

YEASTS/DIRECT-FED MICROBIALS OR PRE/PROBIOTICS

There are many types of yeasts used in premixes. The most commonly used yeast is *Saccharomyces cerevisiae*, a live yeast microbe. Hydrolyzed yeasts and Selenium yeasts are also used as microbial and trace mineral additives. A study by Cagle et al., (2018) illustrated that 10g of Live Yeast (LY) fed daily produced a significant improvement in dry matter digestibility and rumen pH. The study had 4 steers and 4 heifers, all cannulated, randomly separated into one of four treatment groups (CON, LY1= 2.5g, LY2= 5g, LY3= 10g). The experimental units were fed in three stages: a grower, a transitional, and a finisher. The

grower stage did not show any difference when compared to the CON, but the transitional and finisher groups increased dry matter digestibility and rumen pH when fed the 10g of LY.

Adding prebiotics improves digestibility and helps maintain a good balance in the microbe population. Different populations colonize as the diet and type of supplementation changes (Uyeno et al., 2015). Uyeno et al., (2015) illustrates that even with studies showing that prebiotics and probiotics achieve positive balance in the GI microbiota, the dynamics and functions of the rumen communities complexities need to be studied more in-depth to learn the different types and functions of the microflora.

Using the correct prebiotic/probiotic is important, unfortunately, there is limited knowledge on the impact of different prebiotic and probiotic mixtures on microbial populations. Beauchemin et al., (2003) conducted a study on the effect of bacterial direct fed microbials and yeast on digestion, blood, and ruminal acidosis was conducted at a feedlot with eight cannulated steers. The study would determine if a high-concentrate diet could be better utilized and reduce acidosis when given *Saccharomyces cerevisiae*, a common yeast additive, as well as a lactic-acid producing bacterium, *Enterococcus faecium* (EF). The eight cannulated steers had already been exposed to the high-concentrated diet before going into the experiment. Experiment one fed EF and experiment two fed EF, plus the yeast. Experiment one showed an increase in propionate and fecal coliform numbers, while decreasing butyrate, rumen pH, nitrogen, and NDF digestion. All the decreases are considered unfavorable in a ruminant animal. Experiment two increased dry matter digestion of the corn but did not alter the site or extent of nutrient digestion (Beauchemin et al., 2003). Overall, the effect of EF alone or with the yeast did not reduce acidosis or improve the digestion of a high-concentrated diet in cattle in a feedlot.

TRACE MINERALS/CHELATE MINERALS

A study by (Rowe et al., 2014) on the effects of inorganic vs organic trace minerals was conducted on bulls. The semen qualities were tested at ejaculation and then frozen and tested after being thawed. The experimental units were nine bulls assigned to inorganic trace minerals and ten bulls assigned to the organic trace mineral. The bulls were individually penned and fed the trace minerals three times a week for the whole duration of the trial. An adjustment period for the trace minerals was set at the halfway mark of the trial period and then every bull was ejaculated via electroejaculation weekly for the remainder of the experiment. The samples were tested with computer-assisted sperm analysis (CASA), then the semen was frozen with liquid nitrogen, thawed, and tested again. Sperm motility and progression were taken and compared to the different treatments. Rowe et al., (2014) states that a parameter that influences bull fertility and the single most important semen quality is sperm motility. The results showed that the organic trace minerals improved sperm motility and count.

To conduct this study, a combination of additives will be fed to determine if the Angus bull gains in weight, has an increase in fertility, and can maintain a healthier digestive system. COMBO is a well-formulated premix that has been calculated with the correct amount of additives for the best results. The information collected on these breeding bulls and the effects of the prebiotic/probiotic compounds and chelated minerals will add to the very minimal information in the area of growth and the development on the reproductive system for breeding bulls.

MATERIALS AND METHODS

Angus bull calves were stratified by age of dam (AOD) and weaning weight into one of two groups, a control (CON) and a treatment group (COMBO). Both groups were fed the same ration mixture except for the ASU Ram premix in the CON being substituted with the proprietary additive (COMBO) in the treatment group. Specific ingredients and amounts of the COMBO blend will not be disclosed in this report due to confidentiality agreements. Rations were mixed at the Angelo State University feed mill and formulated using the NRC requirements (NRC, 2016). Both groups were given *ad libitum* feed and water along with sufficient cover from the elements during the trial period. Feed was placed into a 4-ton feeder where it was checked daily and replenished as needed. The spring-born Angus bull calves were a part of the purebred Angus herd at the Angelo State University Management Instruction and Research Center (MIR). All research protocols were approved by the Angelo State University Institutional Animal Care and Use Committee (IACUC #19-201).

The bull calves were evaluated for weight gain, scrotal circumference, semen quality, and average daily gain. A factorial arrangement was used to conduct the study and the data collected was analyzed using the proc mixed procedures of SAS. Bull calves were weaned on day -38 and were adapted to concentrate diets using the ASU feed ration CON with weights being taken on day -35 and day -6. The bull calves, CON (n=12), and COMBO (n=13) were separated into their assigned groups on day 0 and fed the experiment rations. Weight and scrotal circumference were measured monthly and recorded on days 0, 29, 58, 102, 115. Weight measures were analyzed as a repeated measure with the AR-1 covariant structure being used. The pdiff option of SAS was used to detect differences in least-squares means. All treatment effects were considered different when ($P \leq 0.05$).

Semen samples were collected via electroejaculation on day 115. Samples were analyzed by the Quick Check Gold Bull Test which takes the freshly ejaculated semen and dilutes the semen with warm media. The semen sample was then taken up with a special syringe to be inserted into the Quick Check to be analyzed and recorded. Then a visual motility score was assessed by a qualified person as a verification standard using a grading scale according to Table 1.

Table 1. Semen grading scores for semen motility parameters

Scale	Grade	Characteristics
5	(+++++) Excellent	More than 80% of the sperm show vigorous motion. Swirls are formed due to the movements of the sperm. The movements are rapid and changing and hard to observe individual sperm samples in undiluted semen.
4	(++++) Very good	About 70-80% of the sperm show vigorous motion which causes waves and eddies but not as vigorous as the excellent grade.
3	(+++) Good	About 45-70% of the sperm are in motion. Motion is vigorous. Waves and eddies are formed slow across the sample.
2	(++) Fair	30-40% of the spermatozoa are in motion. Movements are vigorous. No waves or eddies present.
1	(+) Poor	Little to no mobility found. < 20% of the spermatozoa are in motion. Not progressive and little oscillation.

Adapted from Hossain et al. (2012). This table illustrates the measure of motility of semen samples presented in this report.

RESULTS AND DISCUSSIONS

DESCRIPTIVE STATISTICS

The descriptive statistics of the overall collected data combined in its specific category to calculate a mean with a standard deviation from the mean shown in Table 2. While not all results yield significant levels of variation, many of the variables analyzed were noted as being different.

Table 2. Descriptive statistics of overall collected data

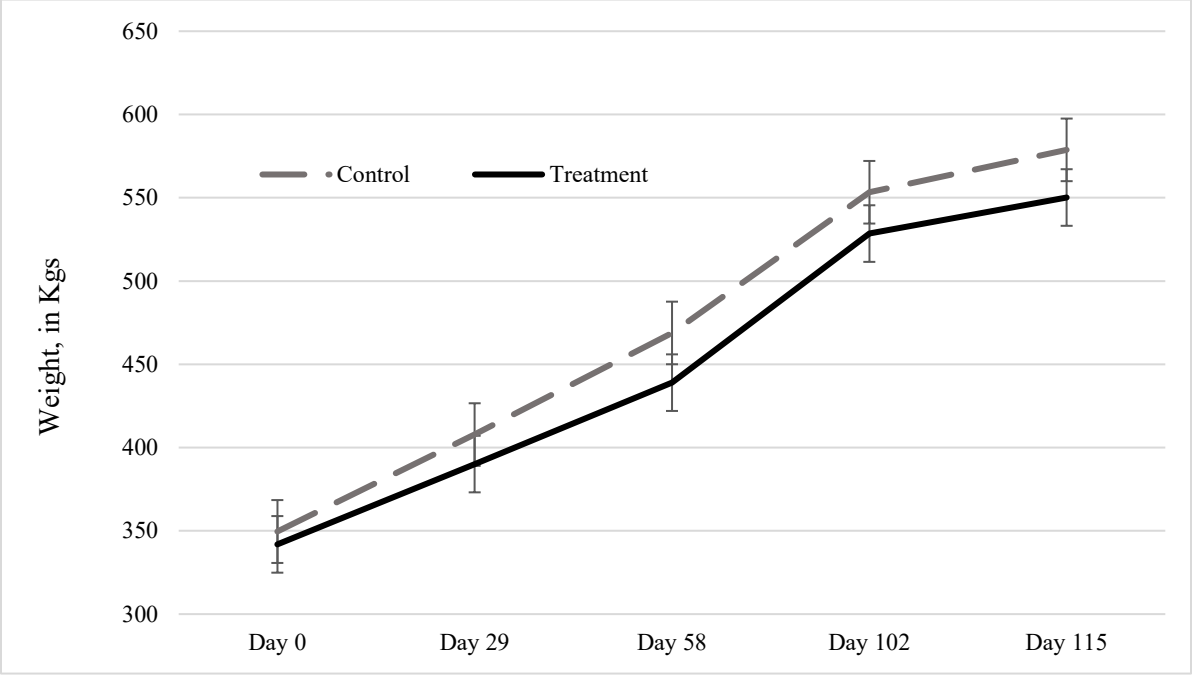
	Mean	Std Dev	Minimum	Maximum
Day 115 Sperm Concentration[1]	287.63	183.71	0	536.7
Day 115 Sperm Motility[2]	49.23	38.18	0	96.5
Day 115 Progressive Motility[3]	32.43	30.2	0	81.5
Day 115 Morphology[4]	73.21	14.79	40.8	88.8
Day 115 Motile Sperm Cells[5]	138.58	139.75	0	499.1
Day 115 Progressive Motile Sperm Cells[6]	88.2	101.16	0	315.8
Day 115 Sperm Cell Velocity[7]	20.48	19.29	0	64
Day 115 Visual Sperm motility scores[8]	3	1.68	0	5
Period 1 Average Daily Gain (ADG) (Day 0 - Day 58), in kg	1.88	0.46	1.05	2.85
Period 2 Average Daily Gain (ADG) (Day 58 - Day 115), in kg	1.99	0.31	1.58	2.63
Scrotal Circumference (Day 58), in cm	36.01	2.63	30.5	40
Scrotal Circumference (Day 115), in cm	37.27	2.49	33.75	41

[1]Million sperm cells/mL
[2]Percent motility
[3]Percent of forward progressing motile sperm
[4]Percent of developed sperm cells
[5]Million cells per mL of semen
[6]Million forward progressing cells per mL of semen
[7]Micron per second
[8]Trained personnel scoring system

BULL WEIGHT

The interaction of treatment × day on body weight is shown in Figure 1.

Figure 1. Weight analysis in response to diet



While no differences due to treatment were observed within any day of the trial, Table 2 illustrates the iso-caloric and iso-nitrogenous nature of the project diets. Because it appears that the CON group maintains a greater rate of weight gain over time, average daily gain (ADG) calculations were evaluated and are presented in Table 3. While ADG data was presented across months, measures were calculated between each weight collection day and a bi-monthly analysis was conducted and was presented in this report for the purposes of clarity and appropriate data interpretation. Differences due to the treatment diet as a main effect was a source of variation in this data as CON fed bulls gained 0.39 kg more weight per day in Period 1 (Day 0 – Day 58) ($P = 0.05$). Microbial populations change as diets change

and different populations colonize as the diet and type of supplementation change (Uyeno et al., 2015). Further analysis of ADG measures revealed that Period 2 (Day 5 – 115) ADG found no significant differences due to diet.

Table 3. Average daily gain by period

	Control	Treatment	SEM	*P-value*
Period 1 (Day 0 - 58)	2.04 lbs.	1.65 lbs.	0.19	0.05
Period 2 (Day 58 - 115)	2.03 lbs.	1.9 lbs.	0.15	0.8

SCROTAL CIRCUMFERENCE

These data suggest that long duration feeding strategies that include this particular dietary treatment combination has a limiting impact on scrotal development while having no impact in overall growth performance. The specific mechanisms that lead to this physiological interruption are currently unknown due to the limited information available related to sources of ingredients, specific quantities of ingredients included, and the unknown mode of action of the combined effects of this diet additive on growing bull scrotal growth. Observations of Rusk et al. (2002), indicate that bulls with a larger scrotal circumference (SC) also exhibit increased yearling body weight and improved fertility. Gipson et al. (1985) found a correlation between SC and the percent live sperm, sperm concentration, and motility as well as potential breeding efficiency score. The least squares means and p-value for scrotal circumference between the control and treatment is shown in Table 4.

Table 4. Scrotal circumference by period

	Control	Treatment	SEM	*P*-value
Day 58	36.83cm	35.63cm	1.32	0.38
Day 115	38.65cm	36.32cm	1.07	0.04

SEMEN SAMPLE ANALYSIS

An evaluation of a breeding bull's ability to successfully mate with and breed multiple females in estrous is routinely evaluated with an annual breeding soundness exam (BSE). A BSE includes the visual assessment of a bull's health and physical mating ability, an evaluation of a bull's reproductive anatomy, and a microscopic visual assessment of a semen sample. Sperm cell concentration and sperm cell motility are standard visual scores and can impact a breeding bull's ability to pass or fail a breeding soundness exam. Sperm concentration is considered a factor of semen quality (Shelke and Dhami, 2001; Belorkar et al., 1988). No differences in sperm concentration were observed in this data (P = 0.79), and therefore the COMBO additive does not appear effect the quantity of sperm cells that develop and maturate prior to ejaculation.

Since sperm cell concentration proved to be adequate, sperm cell motility was expected to be of good value as well. Prior research efforts support that sperm concentration measures positively influences sperm cell motility observations (Everett et al., 1978; Mathevon et al., 1998). Dietary supplement is a significant source of variation for sperm cell motility in this data, ($P = 0.009$). Where CON bulls exhibited 72.5% motility of their total sperm cells in ejaculate samples while 31.09% of the total sperm cells in the COMBO fed

bulls were motile. Further analysis of Table 5 suggests that the progressive motility measure (percent of forward progressing sperm cells), motile sperm cells (million motile sperm per mL of semen), and visual motility scores (Table 1 for scoring parameters) were concerning, but not different statistically. Progressive motility is an assessment of forward progressing sperm cells and is an indicator of fertilization potential (Li et al., 2016). The motile sperm cells data also suggests a biological impact on sperm cell viability in production scenarios as the CON fed bulls produced two times the number of total motile sperm cells. Additionally, as a secondary measurement to the semen analyzer, trained personnel visually assessed the sperm cell motility at 40× magnification and assigned visual scores in a similar methodology that would be used in a breeding soundness exam. Again, although this difference failed to be different in statistical terms, the biological impacts in a beef cattle breeding system warrants extreme caution. Multiple semen quality variables were measured in this study via a Quick Check Test Gold semen analyzer and are summarized in Table 5.

***Table* 5.** Day 115 Semen Sample Analysis

	Treatment	Control	SEM	*P*-value
Day 115 Sperm Concentration[1]	279.36	301.59	82.8	0.79
Day 115 Sperm Motility[2]	31.09	72.5	14.3	0.009
Day 115 Progressive Motility[3]	23	44.49	12.5	0.1
Day 115 Morphology[4]	76.03	72.43	8.1	0.67
Day 115 Motile Sperm Cells[5]	101.13	202.35	56.7	0.09
Day 115 Progressive Motile Sperm Cells[6]	73.76	112.8	44.4	0.38
Day 115 Sperm Cell Velocity[7]	17.49	23.75	8.72	0.48
Day 115 Visual Sperm motility scores[8]	2.4	3.7	0.68	0.07

[1]Million sperm cells/mL
[2]Percent motility
[3]Percent of forward progressing motile sperm
[4]Percent of developed sperm cells
[5]Million cells per mL of semen
[6]Million forward progressing cells per mL of semen
[7]Micron per second
[8]Trained personnel scoring system

IMPLICATIONS

These data suggest that the COMBO diet additive in developing bull diets did not increase weight gain measures over the CON diet consuming bulls. Fertility measures in this study suggest that the COMBO additive showed to have a negative outcome in the overall semen quality evaluation. While this proprietary feed additive is a promising component for enhanced fed, slaughter cattle health and growth performance, it is not recommended to be incorporated into developing bull diets in its current formulation.

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APPENDIX:

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC #19-201)

ANGELO STATE UNIVERSITY

College of Graduate Studies & Research

Institutional Animal Care & Use Committee

01/20/19

Chase Runyan, Ph.D.
Assistant Professor
Co-Chair IACUC
Department of Agriculture
Angelo State University
ASU Station #10888
San Angelo, TX 76909

Dear Dr. Runyan:

Your proposed project titled, "Evaluation of Prebiotic Compounds and Chelated Mineral Additives on Breeding Bull Growth and Fertility" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective 1-20-2019, and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 19-201. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Steve Brewer, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

VITA

Dionicio Gutierrez, Jr. was born in Gonzales, TX, and lived with his parents and three older sisters. He graduated from Dublin High School in 2000 and immediately joined the United States Marine Corps. He served for four years and participated in Operation Iraqi Freedom in 2003. After his service, he started his career as a diesel technician for the next 12 years, which later opened the opportunity for him to begin his formal education. He received a Bachelor of Science in Animal Science with a minor in Range and Wildlife Management from Angelo State University in 2019. He will complete his Master of Science in Animal Science from Angelo State University in May of 2021. He plans to get a job in an agricultural field and contribute to the agricultural industry by applying the knowledge learned at Angelo State University to the best of his abilities.